Exhibit A

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BACKGROUND: Human polymerized hemoglobin (PolyHeme, Northfield Laboratories, Evanston, IL) is a universally compatible, immediately available, disease-free, oxygen-carrying resuscitative fluid being developed as a red cell substitute for use in urgent blood loss. PolyHeme should be particularly useful when red cells may be temporarily unavailable. This article assesses survival at life-threatening RBC hemoglobin concentration ([Hb]) in massively bleeding patients who do not receive red cells.

STUDY DESIGN: There were 171 patients who received rapid infusion of 1 to 20 units (1,000 g, 10 L) of PolyHeme in lieu of red cells as initial oxygen-carrying replacement in trauma and urgent surgery. The protocol simulated the unavailability of red cells, and the progressive fall in RBC [Hb] in bleeding patients was quantified. Thirty-day mortality was compared with a historical control group of 300 surgical patients who refused red cells on religious grounds.

RESULTS: A total of 171 patients received rapid infusion of 1 to 2 units (n = 45), 3 to 4 units (n = 45), 5 to 9 units (n = 47), or 10 to 20 units (n = 34) of PolyHeme. Forty patients had a nadir RBC [Hb] ≤3 g/dL (mean, 1.5 ± 0.7 g/dL). But total [Hb] was adequately maintained (mean, 6.8 ± 1.2 g/dL) because of plasma [Hb] added by PolyHeme. The 30-day mortality was 25.0% (10/40 patients) compared with 64.5% (20/31 patients) in historical control patients at these RBC [Hb] levels.

CONCLUSIONS: PolyHeme increases survival at life-threatening RBC [Hb] by maintaining total [Hb] in the absence of red cell transfusion. PolyHeme should be useful in the early treatment of urgent blood loss and resolve the dilemma of unavailability of red cells. (J Am Coll Surg 2002;195: 445–455. © 2002 by the American College of Surgeons)

The critical issues in resuscitation from acute blood loss in trauma and surgery are restoration of total blood volume and maintenance of sufficient oxygen-carrying capacity to avoid inadequate delivery of oxygen to tissues.[1-4] Inadequate volume replacement leads to a fall in blood pressure and eventual hypovolemic shock. Insufficient red cell replacement might lead to critical levels of anemia, irreversible ischemia, and death.[5,6] Current resuscitation involves initial asanguineous volume replacement with salt solutions, followed by red cell transfusions when compatible blood is available and ad-

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Abbreviations and Acronyms

ASA = American Society of Anesthesiologists
[Hb] = Hemoglobin concentration
P_{50} = Oxygen tension at 50% hemoglobin saturation

equate in supply.[1,2] The goals of treatment are to restore sufficient blood volume to maintain a mean arterial pressure above 60 mmHg, and to replace sufficient red cells to maintain a circulating hemoglobin level >6 g/dL according to the American Society of Anesthesiologists (ASA),[7] or between 7 and 10 g/dL according to the National Institutes of Health (NIH) Consensus Conference.[8] But there are occasions when red cells are temporarily unavailable, inadequate in supply, or cannot be used because of incompatability or religious objection. This might lead to urgent, life-threatening situations, with reported mortality rates of 50% to 95% for hemoglobin levels of ≤3 g/dL.[9-14] An alternative oxygen carrier that could provide immediate life-sustaining therapy until adequate red blood cell hemoglobin levels (RBC [Hb]) could be restored would be clinically useful.

Human polymerized hemoglobin (PolyHeme, Northfield Laboratories Inc, Evanston, IL) is a universally compatible, immediately available, disease-free, oxygen-carrying resuscitative fluid intended for use in the treatment of urgent hemorrhage attendant to trauma and surgery.[15] The goal was to develop a preparation that could be infused rapidly and in massive quantities and would avoid the toxicities observed historically with unmodified hemoglobin[16-19] and in recent trials with modified hemoglobins,[20-23] such as vasoconstriction and renal, pancreatic, gastrointestinal, and cardiac dysfunction. The small molecular-weight tetrameric species of hemoglobin have been associated with these unacceptable adverse effects. The preparation of PolyHeme is designed to avoid these toxicities, and involves polymerization with glutaraldehyde and subsequent purification to remove all unpolymerized tetramer. Early experience demonstrated that infusion to six units (300 g, 3 L) of PolyHeme in urgent blood loss was well tolerated and did not elicit these untoward effects.[24,25]

The present study was designed to evaluate the efficacy of PolyHeme in patients with more dramatic blood loss. The allowable dose was increased in stepwise fashion to a maximum of 20 units (1,000 g, 10 L), equivalent to 2 blood volumes, in exsanguinating patients who received PolyHeme in lieu of red cells as their initial oxygen-carrying replacement. The protocol simulates the unavailability of red cells, and permits the progressive fall in RBC [Hb] levels in bleeding patients to be quantified. This study assesses the ability of PolyHeme to sustain life during rapid, massive hemorrhage in patients with life-threatening RBC [Hb] levels who do not receive red cells.

Table 1. Characteristics of PolyHeme

Index	Measurement
Volume	500 mL
Mass hemoglobin	50 g
[Hb]	10 g/dL
P_{50}	26–32 mmHg
Met[Hb]	<8.0%
Tetramer	≤1.0%
$t_{1/2}$	24 h
Shelf-life	>1 y

[Hb], hemoglobin concentration; P_{50}, oxygen tension at 50% hemoglobin saturation; $t_{1/2}$, half-life.

METHODS

Study design and PolyHeme population

This cohort study compares 30-day mortality in patients receiving PolyHeme with a historical control group of patients who declined blood transfusion for religious reasons. Male and female patients at least 18 years of age were eligible based on the following inclusion criteria: urgent blood loss from trauma or surgery, clinical decision for urgent transfusion in anticipation of low [Hb], or systolic blood pressure less than 100 mmHg from blood loss. Exclusion criteria included: severe head trauma (Glasgow Coma Scale score ≤8), lack of acute blood loss, signs or symptoms of severe organ dysfunction, or pregnancy. The study was conducted at American College of Surgeons certified Level I trauma centers and tertiary care referral institutions. Protocols and consent forms were approved by the Institutional Review Board at each site. Informed consent was obtained from either the patient or an appropriate surrogate.

Description of hemoglobin solution

PolyHeme is a sterile, pyrogen-free, isotonic, and isooncotic solution. The physiologic properties are summarized in Table 1. After lysis of human red cells, pyridoxal phosphate is used to obtain an oxygen tension at 50% hemoglobin saturation (P_{50}) of 26 to 32 mmHg that is elevated compared with the normal red cell P_{50} of 26 mmHg. The tetrameric hemoglobin is polymerized

using glutaraldehyde and any unpolymerized tetramer is then removed. One unit of PolyHeme is composed of 50 g of hemoglobin in 500 mL (10 g/dL) containing sodium chloride, potassium chloride, and pyrogen-free water. This is equivalent to the mass of hemoglobin functionally delivered in a 1-unit red cell transfusion. PolyHeme has a shelf-life of greater than 1 year at 2 to 8°C.

Experimental protocol

All PolyHeme patients received the same treatment and were pooled for analysis, although they were part of three separate FDA-reviewed protocols. Once enrolled, patient care followed Advanced Trauma Life Support[2] and American Association of Blood Banks[26] guidelines on fluid and transfusion therapy, except that PolyHeme was infused in lieu of red cells when a clinical decision was made that transfusion of oxygen-carrying therapy was indicated. All patients received infusions of 1 to 2 L of crystalloid as their initial volume replacement. The indication for and rate of infusion of PolyHeme depended on the patient's clinical status. Patients were eligible to receive up to 1, 3, 6, 10, and finally 20 units (1,000 g, 10 L) of PolyHeme as the allowable dose escalated. If patients required additional oxygen-carrying therapy after the maximum dose of PolyHeme was reached, red cells were transfused as indicated. Clotting factors and platelets were administered when indicated after high-volume blood loss. PolyHeme was infused preoperatively, intraoperatively, and postoperatively to awake and anesthetized patients in emergency rooms, operating rooms, and intensive care units.

Historical control population

Because the goal was to assess the life-sustaining capability of PolyHeme when red cells were unavailable, an appropriate control group would have been concurrent patients receiving only asanguineous fluids. This was not possible because it would have been unethical to withhold oxygen-carrying therapy from patients with such extreme blood loss.[27,28] So we used a historical control group composed of consecutive surgical patients with hemoglobin levels 8 g/dL or less who declined blood transfusion for religious reasons.[29]

Measurements

Hemoglobin determinations

As shown in the equation below, after infusion of PolyHeme, the total hemoglobin is equal to the sum of the hemoglobin carried by the red cells and by the PolyHeme in the plasma.

$$\text{Total [Hb]} = \text{RBC [Hb]} + \text{Poly [Hb]} \quad (1)$$

Plasma and red cells were separated to quantify the hemoglobin carried by each component. Total [Hb] and plasma [Hb] were determined on whole blood and plasma samples, respectively, using automated cell counters. The RBC [Hb] was derived from the hematocrit (Hct), measured by the cell counter, as Hct/3. It was, therefore, possible to document the progressive fall in RBC [Hb] that occurs from ongoing blood loss not treated with red cell replacement, and observe the impact of the PolyHeme in the plasma in maintaining total [Hb].

Mortality

The cumulative 30-day mortality and the 95% confidence intervals are shown for all patients, and for each category of RBC [Hb] for both the PolyHeme recipients and the historical controls.

Statistical analysis

General statistical presentations

The mean and standard deviation are reported for continuous variables, and the frequency and percent are reported for nominal variables.

Mortality comparison

Logistic regression was used to analyze mortality. Terms for treatment group (PolyHeme or historical control), nadir RBC [Hb], and their interaction were used. The interaction between RBC [Hb] and treatment group is considered appropriate for analysis of mortality because at sufficiently high [Hb] the patient will not be at risk of death from anemia, and as [Hb] decreases the risk of death is expected to increase. Patients whose RBC [Hb] was >8 g/dL were not included because they were not at risk of death from anemia.

A supportive analysis was run to test if any differences observed could be because of demographic or medical characteristic parameters. The parameters available in the treatment and historical control groups were age, ASA physical status score, cardiovascular risk, gender, hospital, race, and year of operation. Each of these parameters with their interaction with RBC [Hb] and treatment was tested separately in preliminary models. Subsequently, all significant demographic parameters were included in a full logistic regression analysis.

Table 2. Characteristics of Patients Who Received PolyHeme

Characteristic	Patients
Age (range), y	40.5 ± 17.2 (17 to 85)
Gender	
Male	122 (71.3%)
Female	49 (28.7%)
Race	
Caucasian	95 (55.6%)
Hispanic	49 (28.7%)
African-American	19 (11.1%)
Asian	6 (3.5%)
Native American	2 (1.2%)
Etiology of blood loss	
Blunt trauma	86 (50.3%)
Penetrating trauma	41 (24.0%)
Nontraumatic	44 (25.7%)
Injury Severity Score (range)	17.1 ± 10.4 (1 to 45)

Plus-minus values represent means ± standard deviation.

RESULTS

PolyHeme recipients

Patient characteristics

The 171 patients ranged in age between 17 and 85 years. Other characteristics are shown in Table 2.

PolyHeme infusion

The dose of PolyHeme received by the 171 patients is shown in Table 3. The maximum rate of infusion was approximately 2 units (100 g, 1 L) per minute in uncontrolled hemorrhage. There were no clinically significant safety issues related to the infusion of PolyHeme.

Hemoglobin data

The hemoglobin relationships are demonstrated in Figures 1, 2, and 3. The increase in plasma [Hb] with increasing doses of PolyHeme is shown in Figure 1. The maximum plasma [Hb] was 8.0 g/dL in a single patient who received 8 units (400 g, 4 L) of PolyHeme. The maximum mean plasma [Hb] was 5.9 ± 1.1 g/dL in the group of patients who received 20 units (1,000 g, 10 L) of PolyHeme, reflecting the equilibrium between ongoing blood loss and replacement.

The relationship between total [Hb] and RBC [Hb] for all patients is shown in Figure 2. The figure illustrates that as RBC [Hb] falls from progressive hemorrhage without red cell transfusion, total [Hb] is maintained in the 7 to 10 g/dL range by the infusion of PolyHeme. With hemorrhage requiring more than six units (300 g, 3 L) of PolyHeme, the RBC [Hb] falls below the life-threatening level of 3 g/dL.

There were 40 patients who had an RBC [Hb] ≤ 3 g/dL. Figure 3 shows the total [Hb] for each individual patient as the sum of RBC [Hb] and plasma [Hb]. The patient on the far right had an RBC [Hb] of 0.2 g/dL, with a total [Hb] of 7.5 g/dL. Twenty-nine patients had total [Hb] ≥6 g/dL. Of the 11 patients with total [Hb] <6 g/dL, only two patients received the full 20-unit dose (1,000 g, 10 L). All patients had total [Hb] considerably greater than the critical 3 g/dL level.

Table 3. Dose of PolyHeme Injection Infused (n = 171)

Units	Mass (g)	Volume (L)	Recipients, n
1–2	50–100	0.5–1.0	45
3–4	150–200	1.5–2.0	45
5–9	250–450	2.5–4.5	47
10–20	500–1,000	5.0–10.0	34

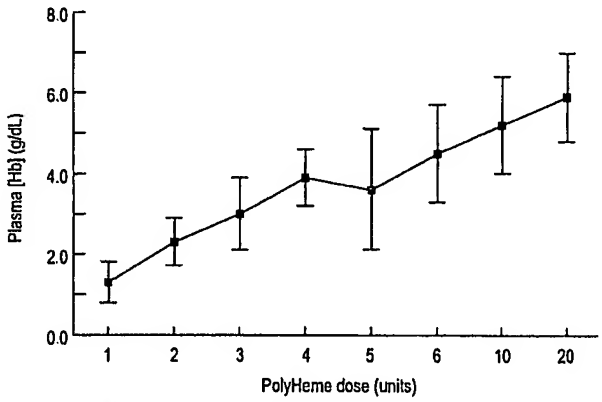

Figure 1. Mean (±SD) plasma [Hb] versus dose of PolyHeme. One unit of PolyHeme contains 50 g hemoglobin in 0.5 L. [Hb], hemoglobin concentration.

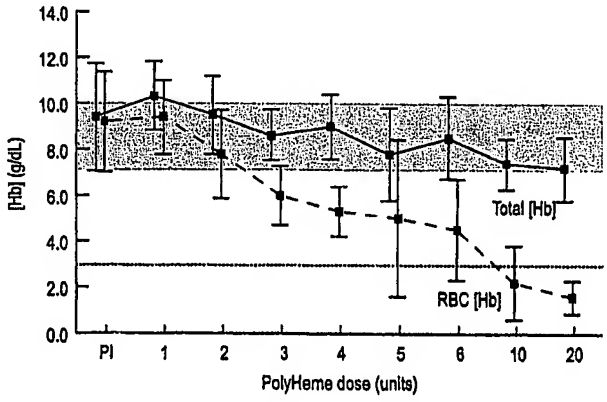

Figure 2. Mean (±SD) total [Hb] and RBC [Hb] versus dose of PolyHeme. Blue shaded area represents 7–10 g/dL guideline according to NIH Consensus Statement. Dotted line represents life-threatening level of 3 g/dL. One unit of PolyHeme contains 50 g hemoglobin in 0.5 L. [Hb], hemoglobin concentration.

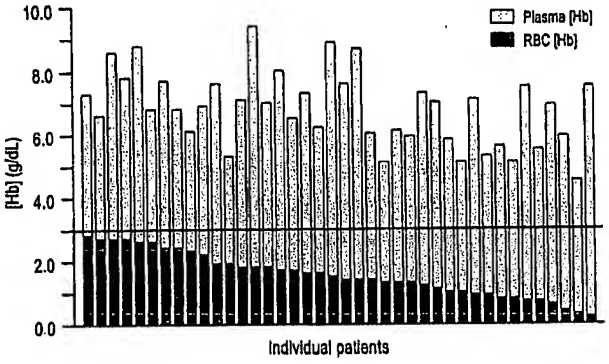

Figure 3. Individual patient data for 40 patients with nadir RBC [Hb] ≤3 g/dL, showing the total [Hb] as the sum of RBC [Hb] (■) and plasma [Hb] (▨). Red line represents life-threatening level of 3 g/dL. [Hb], hemoglobin concentration.

PolyHeme mortality

Mortality data are shown in Table 4. There were 18 deaths overall out of 171 patients, for a mortality of 10.5%. Although mortality increased as RBC [Hb] fell, there was no further increase as the RBC [Hb] fell below 3 g/dL. Nine of the 12 patients with RBC [Hb] ≤1 g/dL survived.

Ten deaths occurred at an early stage (day 0 to day 1), all from exsanguination, including one that was the result of an unknown preexisting liver disease that compromised hemostasis. Three deaths occurred at an intermediate stage (day 1 to day 7). One was from exsanguination, and the other two were from the presenting injury. Five deaths occurred at a late stage (day 7 or later). Four were from multiple organ failure, and one was from complications from preexisting pulmonary fibrosis. None of the deaths was considered due to PolyHeme.

Table 5. Characteristics of Historical Control Patients

Characteristic	Patients
Age (range), y	57 ± 17.2 (18 to 90)
Gender	
Male	89 (29.7%)
Female	211 (70.3%)
Operation type	
Emergency	57 (19%)
Intraabdominal, intrathoracic, or aortic	196 (65%)

Plus-minus values represent means ± standard deviation.

Table 4. Cumulative Mortality by Nadir Postinfusion RBC Hemoglobin Level in PolyHeme Recipients (n = 171)

RBC [Hb] (g/dL)	Deaths/ patients	Mortality (%)	95% Confidence interval (%)
≤12.0	18/171	10.5	6.4 to 16.1
≤11.0	18/170	10.6	6.4 to 16.4
≤10.0	18/165	10.9	6.6 to 16.7
≤9.0	18/158	11.4	6.9 to 17.4
≤8.0	16/150	10.7	6.2 to 16.8
≤7.0	15/133	11.3	6.5 to 17.9
≤6.0	14/100	14.0	7.8 to 22.2
≤5.0	14/84	16.7	9.3 to 26.1
≤4.0	13/62	21.0	11.5 to 32.7
≤3.0	10/40	25.0	12.7 to 41.2
≤2.0	8/30	26.7	12.3 to 45.9
≤1.0	3/12	25.0	5.5 to 57.2

Historical controls

Patient characteristics

The 300 patients ranged in age between 18 and 90 years.[29] Other characteristics are shown in Table 5.

Historical mortality

Table 6 shows the mortality data for the 300 historical control patients.[29] There were 48 deaths overall, for a mortality of 16.0%. There is a marked increase in mortality as RBC [Hb] falls, with no survivors at RBC [Hb] of ≤2 g/dL.

Mortality comparison

The logistic regression produced two different curves (Fig. 4), one for each treatment group. Although mortality increased in both groups as RBC [Hb] decreased, mortality in the PolyHeme group was lower than the historical controls at all RBC [Hb] levels below 7.3 g/dL. This reduction reached significance at all RBC [Hb] levels below 5.3 g/dL ($p < 0.05$).

Table 6. Cumulative Mortality by Nadir Postinfusion RBC Hemoglobin Level in Historical Controls (n = 300)

RBC [Hb] (g/dL)	Deaths/ patients	Mortality (%)	95% Confidence interval (%)
≤8.0	48/300	16.0	12.0 to 20.6
≤7.0	48/201	23.9	18.2 to 30.4
≤6.0	43/145	29.7	22.4 to 37.8
≤5.0	38/91	41.8	31.5 to 52.6
≤4.0	27/59	45.8	32.7 to 59.3
≤3.0	20/31	64.5	45.4 to 80.8
≤2.0	7/7	100.0	59.0 to 100.0
≤1.0	—	—	—

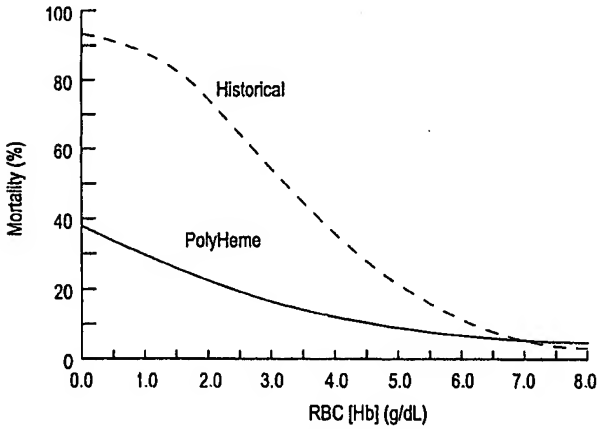

Figure 4. Logistic regression showing mortality in patients who received PolyHeme and in historical controls. Mortality increases in both groups as RBC [Hb] falls. Curves begin to separate at RBC [Hb] of 7.3 g/dL and become significantly different ($p < 0.05$) at RBC [Hb] below 5.3 g/dL. [Hb], hemoglobin concentration.

The two groups differed on the following demographic and medical characteristics: age, ASA physical status score, treatment group, nadir RBC [Hb], and their interaction ($p < 0.05$). But supportive modeling determined that none of these parameters altered the outcomes of the original analysis.

DISCUSSION

This study assesses survival in 171 patients with urgent blood loss who received PolyHeme in lieu of red cells as their initial oxygen-carrying replacement. The protocol simulates a setting where red cells are temporarily unavailable or cannot be used, and the data demonstrate improved survival at all RBC [Hb] below 5.3 g/dL. The most striking observations are for the 81 patients who received 5 (250 g, 2.5 L) or more units, particularly the 34 patients who received 10 (500 g, 5 L) to 20 units (1,000 g, 10 L) of PolyHeme. Because the average blood volume in a normal human is approximately 10 units or 5 liters, this experience represents massive transfusion—a one to two total blood volume exchange.[30] The study design provides a unique opportunity to assess the physiologic activity of PolyHeme in urgently bleeding patients in the virtual absence of any circulating red cells. It represents the most stringent set of experimental conditions, but also reflects the manner in which PolyHeme would likely be used in urgent blood loss.

The physiologic consequences of profound anemia are well understood. In a bleeding but otherwise healthy surgical patient, cardiovascular compensation should be adequate to RBC [Hb] levels of 5 g/dL.[31] As blood loss continues and the RBC [Hb] falls farther, compensatory responses begin to fail[7,8,29,31] and become inadequate when RBC [Hb] falls below 3.5 g/dL,[32-35] with reported mortality rates ranging from 50% to 95% when the RBC [Hb] falls below 3 g/dL.[9-14,29] In this study, 40 patients had RBC [Hb] levels ≤3 g/dL (mean, 1.5 ± 0.7 g/dL), although the total [Hb] was maintained in a therapeutically adequate range (mean, 6.8 ± 1.2 g/dL) by infusion of PolyHeme. Figure 3 documents the substantial [Hb] reserve provided by PolyHeme in each of these individuals at such critically low RBC [Hb] levels. The lowest total [Hb] levels were in patients who had not received the maximum allowable dose of PolyHeme, suggesting additional infusions would have further raised the total [Hb]. The difference between RBC [Hb] and total [Hb] is the physiologic benefit of PolyHeme. The critical finding is the demonstration of the life-sustaining effect of PolyHeme, despite RBC [Hb] ≤3 g/dL.

Of the 171 patients who received PolyHeme, there were 18 deaths (Table 4). The increase in mortality as RBC [Hb] fell likely reflects the more serious injuries causing greater blood loss. Mortality was compared with the 48 deaths in 300 bleeding surgical patients who refused blood because of religious objection. Table 6 documents the progressive increase in mortality in these patients with decreasing RBC [Hb], particularly at [Hb] ≤6 g/dL. The most striking observations are at [Hb] levels ≤3 g/dL. The mortality rate for the historical control patients with RBC [Hb] ≤3 g/dL was 64.5% (20/31 patients), with no survivors at [Hb] ≤2 g/dL. In patients who received PolyHeme and had RBC [Hb] ≤3 g/dL, the mortality rate was 25.0% (10/40 patients).

Perhaps most remarkably, there were 12 patients who received PolyHeme in whom the RBC [Hb] fell to ≤1 g/dL. Nine of these patients survived. The literature does not report any experience at this level because this RBC [Hb] is inconsistent with survival. Figure 4 demonstrates that although mortality increases in both groups as RBC [Hb] falls, the curves begin to separate at [Hb] of 7.3 g/dL and become significantly different below [Hb] of 5.3 g/dL. This observation is consistent with the physiologic observations documenting adequate cardiovascular compensation to [Hb] of 5 g/dL.[31] The improved survival is because of the ability of PolyHeme to maintain an adequate total [Hb] and provide

effective oxygen transport at otherwise lethal RBC [Hb] levels.

A potential criticism of this study is the use of historical data for comparison. An appropriate control group would have been concurrent patients not receiving red cells or any other oxygen-carrying replacement. Such a design would be unethical in settings where red cells were available.[27,28] So we selected the historical comparison and believe it represents a practical and ethical approach to assessing survival in PolyHeme recipients in relation to the expected outcomes in bleeding patients who do not receive red cells. Although there are numerous reports in the literature describing the pooled outcomes in bleeding patients who refuse blood because of religious objection, we have chosen a single study as the basis for comparison because the data are from a single investigator, so the methodology used in collection and analysis of the data is standardized.[29] In addition to being the largest series of patients with [Hb] ≤8 g/dL, the actual individual patient [Hb] measurements are used, unlike the pooled classification in other reports. Common demographic and medical characteristics in the treatment and historical control groups were age, ASA physical status score, cardiovascular risk or history, date of admission, gender, race, and hospital site. Although the two groups differed on all of these parameters, supportive modeling determined that none of these parameters altered the outcome of the original analysis. The important similarity is the progressive blood loss in the surgical setting unaccompanied by red cell replacement. We consider these historical controls valid and useful. Although there have been advances in critical care in recent years, there can be little doubt that with progressive anemia, particularly [Hb] levels ≤3 g/dL, there is a very high mortality rate.

We believe PolyHeme can play an important role as a novel oxygen-carrying resuscitative fluid. It has the potential to simplify and facilitate early treatment of urgent blood loss by permitting immediate, rapid, and simultaneous volume expansion and hemoglobin replacement without red cell transfusion (Fig. 5). The great benefit of PolyHeme is its ability to avoid the onset of life-threatening anemia and subsequent mortality until critical bleeding can be surgically controlled and red cell transfusions are available. Physiologically, PolyHeme loads and unloads oxygen similarly to red cells, the basic requirement for any oxygen carrier.[15,24,36,37] The use of PolyHeme to provide life-sustaining therapy by maintaining adequate total [Hb] during urgent hemorrhage is appropriate, even if the followup period requires subsequent transfusion with red cells. The additional benefits of prolonged storage capability, safety during administration, and the avoidance of clerical errors during such ongoing massive hemorrhage may be substantial and should be considered as part of the value of using PolyHeme in this setting.

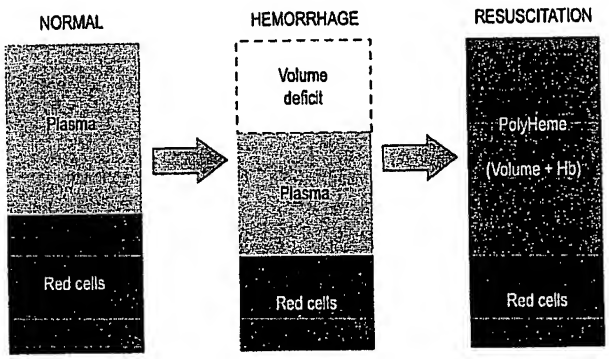

Figure 5. Illustration of the normal blood volume, the changes after a 30% blood volume hemorrhage, and the restoration after resuscitation with Poly SFH-P Injection. (From: Gould SA, Moore EE, Moore FA, et al. J Trauma 1997;43:325–332, with permission)

In summary, this study demonstrates that PolyHeme increases survival at life-threatening RBC [Hb] in massive blood loss in the absence of red cell transfusion. We believe PolyHeme is an effective therapeutic option that should be useful in the early treatment of urgent blood loss, resolving the dilemma of unavailability of red cells.

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Invited Commentary

Ronald V Maier, MD, FACS
Seattle, WA

I congratulate the authors on an intriguing and potentially seminal step forward in the resuscitation of the acutely anemic patient from exsanguinating blood loss. A survival benefit of resuscitation with human polymerized hemoglobin solution in the patient with life-threatening hemorrhage has been a long awaited potential advancement in our armamentarium in the treatment of these critically ill patients. PolyHeme (Northfield Laboratories, Evanston, IL) is native tetrameric hemoglobin polymerized using glutaraldehyde with pyridoxal phosphate added to obtain a normal ox-

ygen affinity and delivery with a P_{50} of 26–32 torr. Previous studies with PolyHeme have confirmed safety after infusion in the clinical setting. This study shows a significant improvement in survival in patients with progressive hemorrhage-induced anemia compared with historical controls. In fact, the historical 100% mortality in patients with red cell hemoglobin less than 2 gm/dL is significantly improved in patients treated with PolyHeme with an associated low 24% mortality. This is truly an impressive improvement in survival.

As the authors point out, it would be unethical to have a true matched control group of patients with progressive anemia that were not transfused. Unfortunately, in the absence of a randomized blinded control study, the use of historical controls raises significant concern for the reliability of the final comparisons. Although one could not envision some improvement not persisting, it is unlikely that the difference is as great as is demonstrated using selected historical control groups. In addition to not only refusing red cells, the historical control patients also refused all coagulation products. In addition to anemia, their survival is further compounded by a persistent coagulopathy, which contributed to their mortality. I would ask the authors to comment on the confounding effect and benefit of correction of the coagulation defect in the PolyHeme group versus uncontrolled bleeding in their control group. In addition, the authors collected extensive posttransfusion data to confirm absence of toxicity, particularly for the well-recognized problems caused by other hemoglobin solutions in renal, hepatic, and cardiac function. Not only was there no evidence of toxicity, but treated patients appear to have done amazingly well considering such a severe insult. Using historical controls based on severity of injury, blood loss, transfusion volume, and other factors, one would predict that the multiple organ failure rate should have been significant in this patient population. Yet in the current study there is *no* organ dysfunction. Using these data one could propose that PolyHeme, in fact, prevents multiple organ failure after severe hemorrhagic blood loss. Will the authors comment on the unexpectedly low rate of organ dysfunction in this group? Is PolyHeme protective against organ dysfunction in this setting of significant ischemia/reperfusion insult and, if so, how?

In addition to carrying oxygen, PolyHeme and other hemoglobin substitutes are known to increase blood pressure from scavenging nitric oxide, an endogenous vasodilator. In fact, clinical trials are currently ongoing to test the beneficial effects of polymerized hemoglobin products as pressors in hemorrhagic and septic shock. Although the authors argue that increased blood pressure and decreased pulse rate in their patients were merely from adequate resuscitation of anemia, it is impossible to discriminate the potential pressor effect of the agent. The increase in mean arterial pressure induced by the polymerized hemoglobin solution from scavenging of nitric oxide might well contribute to the improvement in survival separate from its oxygen-carrying capacity. How did the authors intend to separate these contributions to the clinical outcomes?

Last, although the authors conservatively argue that this new PolyHeme solution could serve well in circumstances of significant blood loss in the absence of available blood, the absence of toxicity and evidence of effectiveness would also argue for its use in the presence of available blood products. The infectious and immunologic impacts of homologous blood transfusion have been increasingly recognized and remain major complicating factors for blood transfusion. One could argue using the current data that PolyHeme should always be used in place of traditional red cell transfusions. Would the authors comment on this enhanced and expanded role for PolyHeme solution? Where do they see its use in the future?

Again, I would like to congratulate the authors on a well-performed study and the college for allowing me to discuss this article.

Reply

Steven A Gould, MD
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I appreciate Dr Maier's kind words supporting our work. He has raised a number of important topics that I will attempt to address. The first issue is the control group. Dr Maier has acknowledged the difficult ethical and logistic issues involved in assessing the impact of PolyHeme on survival in patients who are not transfused. We believe a comparison to patients receiving red cell transfusions does not address the critical issue of survival in the absence of such therapy. We have selected the recently published study by Carson and associates[1] as the

best information available to observe the outcomes of a progressive fall in hemoglobin level in bleeding patients who do not receive blood. We understand the relative limitations of using a study that includes patients who refuse both red cells and coagulation products because of religious objection, unlike our patients who did receive platelets, plasma, and other coagulation factors when necessary. The true difference in survival might actually be as great as demonstrated using these historical data because there is a large body of information from preclinical and clinical studies documenting the excessive mortality that occurs with hemoglobin levels below 3 g/dL.

The issue surrounding coagulopathy is important. Yet the data do not indicate that the control patients died of a persistent coagulopathy. Most of the deaths occurred at a time period well beyond the actual surgery (mean of 8.9 ± 8.4 days), suggesting that it was the low hemoglobin levels and profound anemia that accounted for the mortality rather than coagulopathy. It is clear that the most important therapeutic maneuver in the acutely bleeding trauma patient is surgical control of the hemorrhage and replacement of the lost volume and oxygen-carrying capacity.

Dr Maier has commented on the noteworthy absence of toxicity and organ dysfunction in this study. Other trials with hemoglobin preparations that include a significant quantity of small molecular weight hemoglobin (tetramers) have consistently shown numerous unacceptable adverse events, primarily vasoconstriction and organ dysfunction. We believe that the polymerization and subsequent purification resulting in a tetramer concentration below 1% in our final preparation accounts for the observed ability to rapidly infuse large volumes of PolyHeme and still avoid these toxicities. He has also specifically addressed the issue of multiple organ failure (MOF), which would be expected to occur in such a critically injured group of patients. The described absence of organ dysfunction in our study is stated in terms of the relationship to the infused PolyHeme. There were nine patients (5.3%) in whom MOF did occur. But this remains a low rate compared with what might be predicted in this population, and in no instance did the clinical investigators believe that PolyHeme was responsible. There is a potential scientific basis for this apparent good outcome. Dr Moore and his colleagues in Denver have done considerable work assessing MOF in the trauma population, and have implicated activation of the neutrophil as the principal event in the development of this syndrome.[2-6] One of the main risk factors they have identified for development of MOF in trauma is the transfusion of more than six units of blood in the first 12 hours after injury.[7-10] They have shown that there are multiple components contained in stored blood that result in increased levels of a number of proinflammatory mediators that lead to such neutrophil activation.[8,10-12] They have also published a number of in vitro and in vivo observations documenting the absence of any inciting or priming activity of PolyHeme on the neutrophil.[13-15] Although the clinical studies have not allowed a pure evaluation of this hypothesis, there is an ongoing attempt to collect such data in trauma patients in Denver. This may well represent an exciting outcome in the future.

The issue of scavenging of nitric oxide and vasoconstriction is also an important consideration. We believe that the toxicities described with other hemoglobin preparations are largely from extravasation of small molecular weight hemoglobin that results in the binding of nitric oxide in the interstitial space. This produces the observed vasoconstriction that likely accounts for the accompanying organ dysfunction. We believe that having a tetramer concentration below 1% prevents this sequence from occurring. Dr Maier asked how we separated the observed increase in blood pressure and decreased pulse rate in our patients, considered related to the appropriate volume resuscitation of the patient, from the potential vasoconstriction from the PolyHeme itself. We have several answers to this important question. First, in our Phase I trials, the infusion of one unit (50 gm) of PolyHeme in young, healthy male volunteers, fully awake and instrumented with indwelling radial artery catheters for continuous monitoring, produced absolutely no change in blood pressure, heart rate, or inulin clearance during or after the actual infusion. We believe this is the most sensitive setting to look for such vasoconstriction. In our randomized trial comparing six units of PolyHeme to six units of blood presented at another Papers Session at the 1997 American College of Surgeons meeting and published in the *Journal of the American College of Surgeons*,[16] we documented the absence of any difference in blood pressure or heart rate response during or after resuscitation between patients receiving either blood or PolyHeme. This is the most relevant setting to look for vasoconstriction. We believe these observations allow us to accurately separate the vasoconstrictive effects from the volume expansion effects.

Finally, Dr Maier raises the rather provocative issue of whether PolyHeme should actually be used in place of

red cell transfusions even when they are available. Although this is a flattering suggestion, at the present time we believe the most appropriate initial use for such a novel therapy is when red cells are not available or cannot be used because of immunologic incompatibility or religious objection. PolyHeme delivers the same mass of hemoglobin in each unit and achieves the same increment in oxygen-carrying capacity in the recipient as a unit of red cells, but the intravascular persistence is shorter. In elective surgical settings, which typically involve slower and more modest blood loss, transfusions are often given after the bleeding is under control. The longer lasting red cells are likely to be more appropriate and safe in this setting. We continue to believe the most appropriate role for PolyHeme is in the early management of a patient with urgent blood loss. The immediate availability and universal compatibility are particularly important logistic benefits in such rapid, unplanned hemorrhage. The great physiologic benefit of PolyHeme is its ability to avoid life-threatening hemoglobin levels and subsequent mortality during ongoing massive hemorrhage until surgical hemostasis is obtained and red cell transfusions are available and safe. There might well be other benefits, such as those being studied by Dr Moore and his colleagues, that will eventually make the use of PolyHeme preferable to red cell transfusion in the future. We need to complete those trials before we make such a major transition in our current approach to trauma resuscitation. But that is certainly a potentially exciting outcome for the future.

In summary, Dr Maier has highlighted the key issues surrounding this study. I want to thank him again for his comments, and thank the College for allowing me to present this article.

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